

CLAIMS

1. A method of producing male or female sterile plants comprising the steps of transforming plant material with a polynucleotide which encodes at least one enzyme
5 which reacts with a non-phytotoxic substance to produce a phytotoxic one, and regenerating the thus transformed material into a plant, wherein the said non-phytotoxic substance is applied to the plant up to the time of male or female gamete formation and/ or maturation, so that the non-phytotoxic substance provides for the production of a phytotoxic one which selectively prevents the formation of or
10 otherwise renders the said gametes non-functional, wherein the enzyme is expressed preferentially in either male or female reproductive structures and the non-phytotoxic substance is a D-alpha amino acid, or a peptide derivative of a non-protein D-alpha amino acid, characterised in that the enzyme is a mutant D-amino acid oxidase, obtainable from *Rhodotorula gracilis*, which oxidase comprises a lysine at position 58
15 rather than a phenylalanine in the wild type sequence.
2. A method according to claim 1, wherein the said non-phytotoxic substance is applied in mixture along with at least one further substance which is selected from the group consisting of safeners, gametocides, glutathione-S-transferase inducers, cytochrome
20 P450 inducers, herbicides, fertilizers, nematocides, synergists, insecticides, fungicides, hormones, plant-growth regulators and cytochrome P450 inhibitors.
3. A method according to claim 1 or 2, wherein the non-phytotoxic substance is applied foliarly and is a phloem mobile and metabolically stable oxidisable substrate of the
25 enzyme, wherein the enzyme provides the phytotoxic product, as a direct or indirect one from the non-phytotoxic substance.
4. A method according to the preceding claim, wherein the phytotoxic product is an indirect one produced in the form of peroxide and/or a super oxide anion.
30
5. A method according to either of claims 3 or 4, wherein the non-phytotoxic substance is D-aspartate or D-glutamate and the enzyme oxidises the said amino acid to a 2-keto acid with concomitant reduction of oxygen to a peroxide anion.

6. A method according to any preceding claim wherein the enzyme comprises substitutions at positions 213, 223 and/or 238 when compared to the wild type sequence.
- 5
7. A method according to the preceding claim, wherein the oxidase has at position 213 an amino acid selected from the group consisting of His, Ser, Thr, Cys, Gln, Gly, Asn and Ala, and/or at position 238 an amino acid selected from the group consisting of His, Ser, Thr, Cys, Asn, Gln, Gly and Ala, and/or at position 223 an amino acid selected from the group consisting of His, Ser, Thr, Cys, Ala, Gly, Gln and/or Asn.
- 10
8. A method according to the previous claim where the amino acid at position 213 is Ser or Thr.
- 15
9. A method according to any one of claims 3-8, wherein the enzyme is targeted to other than the peroxisome.
10. A method according to claim 1 or 2, wherein the non-phytotoxic substance is either the D enantiomer of phosphinothricin or a D enantiomer of bialaphos.
- 20
11. A method according to either of claims 1 or 2, wherein the non-phytotoxic substance is comprised within a mixture, which contains a phytotoxic substance and wherein the enzyme oxidises an amino acid to a 2-keto acid with concomitant reduction of oxygen to a peroxide anion.
- 25
12. A method according to the preceding claim wherein the enzyme is a mutant D-amino acid oxidase obtainable from *Rhodotorula gracilis* which oxidase comprises substitutions at positions 213 and/or 238 and/or 223 when compared to the wild type sequence, or is a D-aspartate oxidase.
- 30
13. A method according to the preceding claim, wherein the oxidase obtainable from *Rhodotorula* has at position 213 an amino acid selected from the group consisting of His, Ser, Thr, Cys, Gln, Gly, Asn and Ala, and/or at position 238 an amino acid

- 55 -

selected from the group consisting of His, Ser, Thr, Cys, Gln, Gly, Asn and Ala, and at position 223 an amino acid selected from the group consisting of His, Ser, Thr, Cys, Gln, Gly, Asn and Ala.

- 5 14. A method according to the previous claim where the amino acid at position 213 is Ser or Thr.
- 10 15. A method according to any one of claims 10-13, wherein the mixture comprises both D and L phosphinothricin and the plant material expresses a PAT gene substantially only in green tissues and/or in floral tissue which produce gametes being other than those that are rendered non-functional.
- 15 16. A mutant D-amino acid oxidase obtainable from *Rhodotorula gracilis*, capable of oxidising phosphinothricin, which comprises a lysine at position 58 rather than a phenylalanine in the wild type sequence.
17. An oxidase according to claim 14, further comprising amino acid substitutions in at least one position selected from the group consisting of 213, 223, 238.